Sortase A

Week 6

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Overlap Extension PCR of SortA and GB1 - Trial 1

Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Q5® High-Fidelity 2X Master Mix

Modifications and comments to protocols

No Modifications were made to the protocol.
The total volume of each reaction was set to 25 μl.
Annealing temperature was set to 66 °C

Experimental Data

Table 1: Samples used for the overlap extension PCR and their concentration.

Sample	Concentration [ng/ul]
SortA 1	93.5
SortA 2	105
GB1 (Stock)	10

The expected size of the overlap extension PCR product is 662 bp, where the size of Sortase A is 491 bp and for GB1 171 bp. Thus a band around the size of 700 bp is expected to be observed when gel electrophoresis has been performed.

Table 2: Overlap extension PCR, experimental set-up - Trial 1.

Component	25 μl Reaction	Final Concentration [ng/μl]
10 μM Forward Primer	1.25	0.5 μM
10 μM Reverse Primer	1.25	0.5 μM
Template DNA (SortA 1, SortA 2)	5	-
GB1	5	0.2
Q5 High-Fidelity 2x Master Mix	12.5	1x
Water	0	-

Results and Conclusions

Table 3: The concentration of the overhang extension PCR samples after PCR Clean up, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/ul]
GS 1	23
GS 2	20.7

A band around the expected size of 700 bp can be observed in the gel below, indicating a successful overlap extension PCR. Unspecific bands are also observed on the gel.

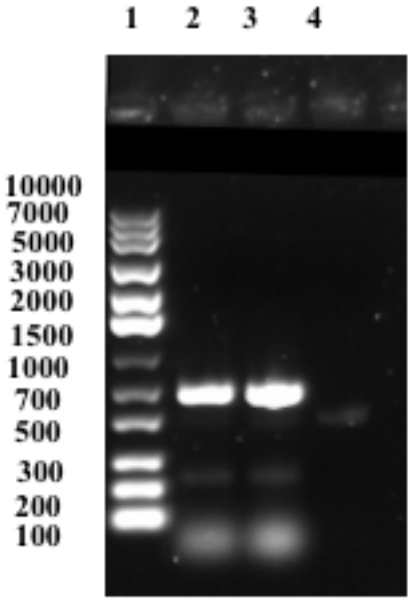

Figure 1: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GB1-SortA 1, (3) GB1-SortA 2, (4)SortA 1. A band for both overlap extension PCR samples of the expected size around 700 bp can be observed on the gel.

Discussion and Troubleshooting

Overlap extension PCR was deemed successful given the clear bands around the expected size on the gel (Figure 1), though there are unspecific bands on the gel. Thus a gel purification of samples should be conducted to ensure samples containing the construct of interest Subsequent steps will focus on amplifying the new construct and restriction cloning in to a vector.

2 Amplification of GB1-SortA

Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Q5® High-Fidelity 2X Master Mix

Modifications to protocols

No Modifications were made to the protocol.
The total volume of each reaction was set to 25 μl.
Annealing temperature was set to 66 °C

Experimental Set-up

The aim is to use Forward primer $Prefix_F$ and reversed primer $SortA_Rev2$, to amplify the whole construct GB1-SortA (GS). Samples GS 1 and GS 2 from **Experiment 1** were used.

Table 4: Experimental set-up for PCR of GS1 and G2

Component	25 μl Reaction	Final Concentration
10 μM Forward Primer	1.25	0.5 μM
10 μM Reverse Primer	1.25	0.5 μM
Template DNA (GS1, GS2)	1	27, 20.9
Q5 High-Fidelity 2x Master Mix	12.5	1x
Water	9	-

Results and Conclusions

Table 5: The concentration of the samples after PCR Clean up, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/ul]
GS 1	170
GS 2	185.6

Unspecific bands at sizes that were not of interest were observed on the gel after electrophoresis.

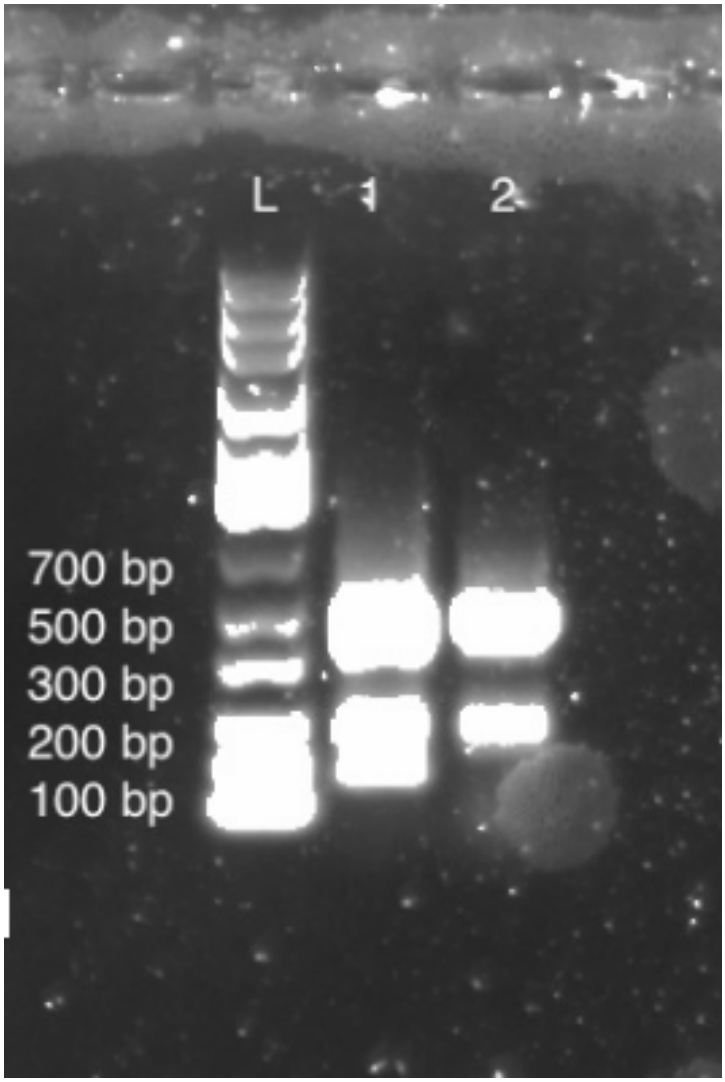

Figure 2: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GB1-SortA 1 amplified, (3) GB1-SortA 2 amplified. Both wells containing the amplified samples show unspecific bands at sizes that are not of interest.

Discussion and Troubleshooting

Analyzing the gel (Figure 2), the conclusion can be drawn that amplification of GB1-SortA construct was unsuccessful. The primers might have annealed on unspecific sites, causing the unexpected bands on the gel. Subsequent experiment will focus on redoing the overlap extension PCR followed by gel purification of samples to avoid contamination of unwanted PCR products.

3 Overlap extension PCR - Trial 2

Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Q5® High-Fidelity 2X Master Mix

Modifications and comments to protocols

No Modifications were made to the protocol.
The total volume of each reaction was set to 50 μl.
Annealing temperature was set to 66 °C

Experimental Data

Samples SortA 1 and SortA 2 were used for the overlap extension PCR. Duplicates were made of each sample named, GS 1.1, GS 1.2, GS 2.1 and GS 2.2.

Table 6: Samples used for the overlap extension PCR and their concentration.

Sample	Concentration [ng/ul]
SortA 1	93.5
SortA 2	105
GB1 (Stock)	10

The expected size of the overlap extension PCR product is 662 bp, where the size of Sortase A is 491 bp and for GB1 171 bp. Thus a band around the size of 700 bp is expected to be observed when gel electrophoresis has been performed.

Table 7: Overlap extension PCR, experimental set-up - Trial 2.

Component	50 μl Reaction	Final Concentration [ng/μl]
10 μM Forward Primer	2.5	0.5 μM
10 μM Reverse Primer	2.5	0.5 μM
Template DNA (SortA 1, SortA 2)	10	-
GB1	1	0.2
Q5 High-Fidelity 2x Master Mix	25	1x
Water	9	-

Results and Conclusions

A band around the expected size of 700 bp can be observed in the gel below, indicating a successful overlap extension PCR. Unspecific bands are also observed on the gel.

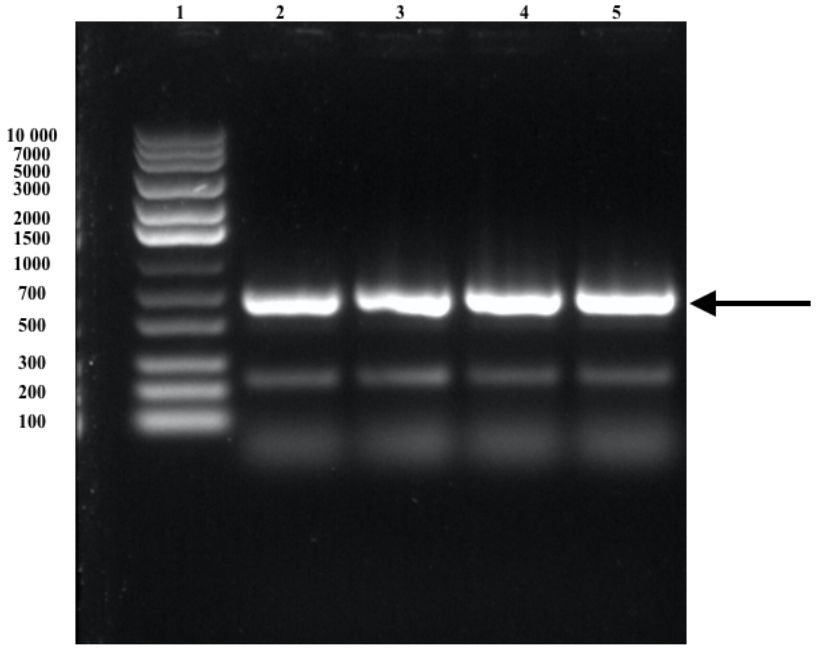

Figure 3: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GS 1.1, (3) GS 1.2, (4) GS 2.1 (5) GS 2.2. A band for all overlap extension PCR samples of the expected size around 700 bp can be observed on the gel.

Samples were gel purified after the electrophoresis to get rid of contaminants from unspecific bands on the gel.

Table 8: The concentration of the overhang extension PCR samples after Gel purification, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
GS 1.1	7.9
GS 1.2	6.6
GS 2.1	3.5
GS 2.2	3

Discussion and Troubleshooting

Overlap extension PCR was deemed successful given the clear bands around the expected size on the gel (Figure 1). Lower concentrations of the samples were apprehended, which was expected given the low yield gel purification generate. Subsequent steps will focus on restriction cloning of the GS construct into a vector.

4 Digestion of GS

Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Digestion and Ligation

Modifications and comments to protocols

No Modifications were made to the protocol.
The total volume of each digest reaction was set to 8 μl.

Experimental Set-up

The table below summarizes the digestions made on GS.

Table 9: Samples used for the overlap extension PCR and their concentration.

Sample	Concentration [ng/ul]
SortA 1	93.5
SortA 2	105
GB1 (Stock)	10

The expected size of the overlap extension PCR product is 662 bp, where the size of Sortase A is 491 bp and for GB1 171 bp. Thus a band around the size of 700 bp is expected to be observed when gel electrophoresis has been performed.

Table 10: Overlap extension PCR, experimental set-up - Trial 2.

Component	50 μl Reaction	Final Concentration [ng/μl]
10 μM Forward Primer	2.5	0.5 μM
10 μM Reverse Primer	2.5	0.5 μM
Template DNA (SortA 1, SortA 2)	10	-
GB1	1	0.2
Q5 High-Fidelity 2x Master Mix	25	1x
Water	9	-

Results and Conclusions

A band around the expected size of 700 bp can be observed in the gel below, indicating a successful overlap extension PCR. Unspecific bands are also observed on the gel.

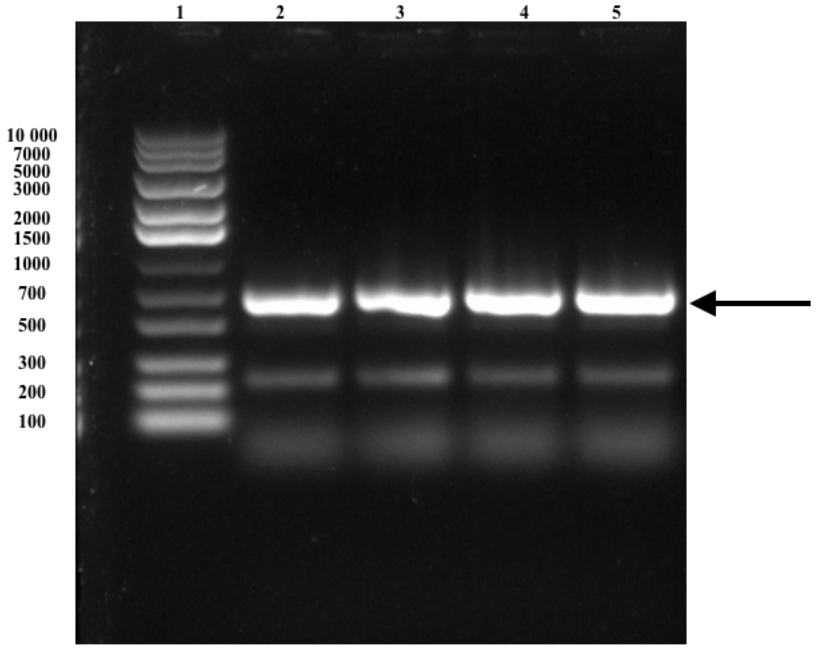

Figure 4: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GS 1.1, (3) GS 1.2, (4) GS 2.1 (5) GS 2.2. A band for all overlap extension PCR samples of the expected size around 700 bp can be observed on the gel.

Samples were gel purified after the electrophoresis to get rid of contaminants from unspecific bands on the gel.

Table 11: The concentration of the overhang extension PCR samples after Gel purification, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
GS 1.1	7.9
GS 1.2	6.6
GS 2.1	3.5
GS 2.2	3

Discussion and Troubleshooting

Overlap extension PCR was deemed successful given the clear bands around the expected size on the gel (Figure 1). Lower concentrations of the samples were apprehended, which was expected given the low yield gel purification generate. Subsequent steps will focus on restriction cloning of the GS construct into a vector.